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Lake Erie *Microcystis*: Relationship between microcystin production, dynamics of genotypes and environmental parameters in a large lake

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ABSTRACT

Cyanobacteria of genus *Microcystis* sp. have been commonly found in Lake Erie waters during recent summer seasons. In an effort to elucidate relationships between microcystin production, genotypic composition of *Microcystis* community and environmental parameters in a large lake ecosystem, we collected DNA samples and environmental data during a three-year (2003–2005) survey within Lake Erie and used the data to perform a series of correlation analyses. Cyanobacteria and *Microcystis* genotypes were quantified using quantitative real-time PCR (qPCR). Our data show that *Microcystis* in Lake Erie forms up to 42% of all cyanobacteria, and that *Microcystis* exists as a mixed population of potentially toxic and (primarily) non-toxic genotypes. In the entire lake, the total abundance of *Microcystis* as well as the abundance of microcystin-producing *Microcystis* is strongly correlated with the abundance of cyanobacteria suggesting that *Microcystis* is a significant component of the cyanobacterial community in Lake Erie during summer seasons. The proportion of total *Microcystis* of all cyanobacteria was strongly linked to the microcystin concentrations, while the percentage of microcystin-producing genotypes within *Microcystis* population showed no correlation with microcystin concentrations. Correlation analysis indicated that increasing total phosphorus concentrations correlate strongly with increasing microcystin concentrations as well as with the total abundance of *Microcystis* and microcystin-producing *Microcystis*.

1. Introduction

Microcystis blooms in the Laurentian Great Lakes, especially in Lake Erie, have been observed almost annually over the past decade (Brittain et al., 2000; Vincent et al., 2004; Hotto et al., 2005; Rinta-Kanto et al., 2005). In the 1960s, cyanobacterial blooms were common in a then severely eutrophied Lake Erie, but reductions in external phosphorus loading implemented in the 1970s reduced cyanobacterial biomass (Makarewicz and Bertram, 1991); by the late 1980s water quality had improved substantially, and algal biomass had decreased up to 89% in off-shore waters (Makarewicz, 1993). Since the mid-1990s, cyanobacterial blooms composed primarily of *Microcystis* spp., have once again increased in frequency, and this has been considered a sign of returning eutrophic conditions in the lake (Conroy et al., 2005). While *Microcystis* spp. are not the sole microcystin-producers in the system (Rinta-Kanto and Wilhelm, 2006), *Microcystis* spp. are abundant and wide-spread compared to other potentially toxic cyanobacteria (Ouellette et al., 2006; Conroy et al., 2007). Microcystin concentrations $>1\ \mu g\ L^{-1}$ have been commonly observed during *Microcystis* blooms in Lake Erie (Brittain et al., 2000; Rinta-Kanto et al., 2005) and thus *Microcystis* has been considered as the major producer of the toxin in the lake.

In natural assemblages *Microcystis* cells exist mostly as colonies held together by a mucilaginous matrix. These colonies can float on the water surface due to the presence of intracellular gas vesicles (Komarek, 2003). A subset of *Microcystis* cells carries a 55 kb microcystin synthetase (*mcy*) gene cluster required for the production of the toxin microcystin, whereas non-toxin producing genotypes generally lack or contain an incomplete copy of this gene cluster and thus lack the ability to produce the toxin (Kaebernick and Neilan, 2001). Microcystins are a chemically diverse group of cyanotoxins which in humans have been known to cause gastroenteritis, liver damage and, in the most severe case,

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the death of 60 hemodialysis patients in Brazil in 1996 (Pouria et al., 1998; Kuiper-Goodman et al., 1999; Sivonen and Jones, 1999). Some evidence of tumor-promoting activity of microcystins also exists (Falconer, 1991; Dietrich and Hoeger, 2005).

Several previous studies have addressed the factors which influence the proliferation and toxin production of *Microcystis*, including examinations of the relationships between limnological factors and the abundance of *Microcystis* in natural assemblages (Wicks and Thiel, 1990; Kotak et al., 2000; Oh et al., 2001; Graham et al., 2004; Giani et al., 2005). Studies of *Microcystis* in natural populations have suggested some of the key factors affecting cell proliferation and toxin production including increased phosphorus loading, the stoichiometric ratio of nitrogen and phosphorus, solar radiation, pH and temperature of the water, primary production and oxygen saturation (Wicks and Thiel, 1990; Kotak et al., 2000), although in complex aquatic systems the conditions that allow development of *Microcystis* blooms and induce toxin production are usually the sum of a variety of factors. While relying on traditional methods for cell quantification, previous studies have addressed the total *Microcystis* population, which in natural assemblages is known to consist of phenotypically identical toxic and non-toxic genotypes (Kurmayer et al., 2002).

PCR-based methods have been commonly used to detect the genotype-specific target genes in samples. The sequence analysis of ribosomal RNA intergenic transcribed spacer (rRNA-ITS) region has also proven useful for distinguishing potentially toxic and non-toxic genotypes of *Microcystis* and thereby for elucidating the dynamics and diversity of the genotypes in natural assemblages (Janse et al., 2004; Janse et al., 2005; Kardinaal et al., 2007). Quantitative real-time PCR (qPCR) has been successfully applied to track quantities of microcystin-producing *Microcystis* genotypes (cells containing one of the *mcy*-genes) in natural populations (Kurmayer et al., 2003; Kurmayer and Kutzenberger, 2003; Vaitomaa et al., 2003; Rinta-Kanto et al., 2005; Yoshida et al., 2007).

So far, the effects of environmental factors on the abundance of microcystin-producing and non-toxic *Microcystis* genotypes have been studied on a relatively limited scale (Yoshida et al., 2007). In order to provide a more comprehensive view on the relationship between the genotypic composition of natural *Microcystis* populations and microcystin concentration, and the relationship of environmental factors to these, we collected DNA, microcystin and nutrient samples from different parts of Lake Erie during the summer seasons of 2003, 2004 and 2005. We applied qPCR to quantify specific marker genes for cyanobacteria, *Microcystis* and microcystin-producing *Microcystis* to quantitatively determine the genotypic composition of the natural *Microcystis* population. Microcystin concentrations were correlated with qPCR data to investigate the linkage between the abundance of *Microcystis* genotypes and microcystin concentrations in Lake Erie across spatial and temporal scales. Furthermore, we also investigated correlations between limnological parameters (such as concentrations of nutrients and water temperature, pH) and microcystin concentrations as well as the abundance of *Microcystis* genotypes in Lake Erie to elucidate the factors that affect the *Microcystis* population dynamics and toxin production in this large lake ecosystem.

2. Materials and methods

Samples used to generate the data for this study were collected from Lake Erie as previously described (Rinta-Kanto et al., 2005) on board the *C.C.G.S. Limnos* and, additionally in August 2003, on board the *R/V Lake Guardian* and a research support craft from Lake Erie Center (Toledo, OH). Samples were collected from 1 m depth using a surface water pump (on board *C.C.G.S. Limnos*) or Niskin bottles (*R/V Lake Guardian*) and samples from deeper than 1 m were collected using Niskin bottles mounted on a rosette sampler. Due to the bathymetry of the lake and more intensive sampling within the shallow western basin, *ca* 75% of the samples were collected at 1 m, with the remaining 25% being collected from depths (2–40 m). Temperature was determined using the deckboard profiler on the *C.C.G.S. Limnos*. Water column pH was collected on deck of the ship after sample collection using a standard lab probe calibrated on site with standard buffer solutions (pH 4.0, 7.0 and 10.0, Fisher Scientific).

Water samples for DNA extraction were collected by filtering known volumes of lake water (typically 30 mL, but adjusted to 5 mL for sites with high biomass) on 0.22-μm nominal pore-size polycarbonate filters (Poretics). Filter funnels were rinsed with 10% sodium hypochlorite solution and distilled water between samples to reduce the potential for cell carryover between samples. Filters were stored frozen (−20 °C) until DNA extraction. DNA extraction was carried out as described previously (Rinta-Kanto et al., 2005).

The abundance of cyanobacterial 16S rDNA copies, *Microcystis*-specific 16S rDNA copies and *mcyD* gene copies were independently determined using the qPCR assays described in detail in Rinta-Kanto et al. (2005) using their primers and probes at a concentration of 0.4 μM (final). The target gene copy abundance and the abundance of cyanobacteria, total *Microcystis* and toxic *Microcystis* cells for each sampling location was the arithmetic mean of triplicate PCR reactions run for each sample. To standardize target gene quantities, single copy insert plasmids were used to establish standard curves as described in the previous study (Rinta-Kanto et al., 2005). We also examined our results after converting our target gene abundance to cell densities based on genomic DNA standards generated from our model cell, *Microcystis aeruginosa* LE-3, which is estimated to contain 2 rRNA operons and one *mcyD* gene per genome. In the results the cell densities are reported as *Microcystis aeruginosa* LE-3 equivalents. To avoid potential bias introduced by the conversion of target gene abundance to cell quantities we have used the absolute quantities of individual target genes in the statistical analysis.

Water column chlorophyll-*a* concentrations were used as a proxy for phytoplankton biomass. Chlorophyll-*a* concentrations were estimated in duplicate samples of lake water filtered on 0.2-μm nominal pore-size polycarbonate filters (47-mm diameter, Millipore) after extraction (*ca* 24 h, 4 °C) in 90% acetone. Chlorophyll-*a* retained on the filters was quantified with either an AU-10 or TD-700 fluorometer (Turner Designs; Sunnyvale, CA) using the non-acidification protocol (Welschmeyer, 1994) and solid standard (Turner Designs; Sunnyvale, CA) normalization to account for inter- and intra-machine variations.

For phycocyanin determinations, discrete water samples (1 L) were collected and filtered onto 47-mm Whatman 934-AH glass fiber filters. Phycocyanin concentrations were estimated fluorometrically using a modification of the method of Abalde et al. (1998) and Siegelman and Kycia (1978). Phycocyanin was extracted from these filters by freezing the samples at −21 °C and thawing at 4 °C three times in 10 mM phosphate buffer (pH 6.8) under dim light. The extract was clarified by centrifugation at 22,000 × *g* for 15 min and the phycocyanin concentration in the supernatant determined by fluorescence using a Turner Designs 10-AU fluorometer equipped with a 577 nm band pass excitation filter and 660 nm cutoff emission filter with a cool white light source.

Microcystin in water samples was determined by means of protein phosphatase inhibition assays. Samples were collected on GF/F (Whatman) filters for toxin extraction as in our previous work (Rinta-Kanto et al., 2005). The assays were run in 96-well plates containing 0.1 mU enzyme (recombinant protein phosphatase 1A, catalytic subunit, Roche Applied Science), 1.05 mg para-nitrophenyl phosphate (Sigma) and 10 μL of sample or microcystin-LR

(Sigma Biochemical) using the method of Carmichael and An (1999). The rate of phosphate hydrolysis was calculated from the change in absorbance at 405 nm over 1 h, and then compared to the control (no added microcystin-LR) and to standards containing between 6 and 40 $\mu g\,L^{-1}$ microcystin-LR. In the results, all reported toxin concentrations are microcystin-LR equivalents. Blanks (no enzyme, no toxin), unknowns, standards, and controls were all run in duplicate.

Total phosphorus (TP) (filtered and unfiltered), soluble reactive phosphorus (SRP), total nitrogen (TN) (filtered), NO_3, NH_3 and SiO_2 were determined from lake water samples in 2004 and in 2005 as described in DeBruyn et al. (2004). Nutrient concentrations in the filtered fraction refer to the dissolved nutrients passing through 0.22-μm nominal pore-size filter, whereas the unfiltered concentrations were measured directly from the lake water.

Total chlorophyll-*a* concentrations (chlorophyll-*a* retained on a 0.2-μm nominal pore-size filter) were calculated as the arithmetic mean of measurements from duplicate samples; microcystin-LR concentration was an arithmetic mean of duplicate measurements from single samples; and phycocyanin concentration, pH, TN (dissolved), NO_3, NH_3, SiO_2, SRP, TP (dissolved and dissolved + -particulate) were results from single measurements. Molar TN:TP ratio was calculated using the molar concentrations of dissolved TN and TP.

Since assumptions of normality were not met in distributions of all variables, the nonparametric Spearman Rank Correlation coefficient was calculated as a measure of correlation between all possible pairs of variables. Statistical analysis was completed using the NCSS statistical analysis software package. Correlations warranting $P < 0.05$ were considered significant in this analysis.

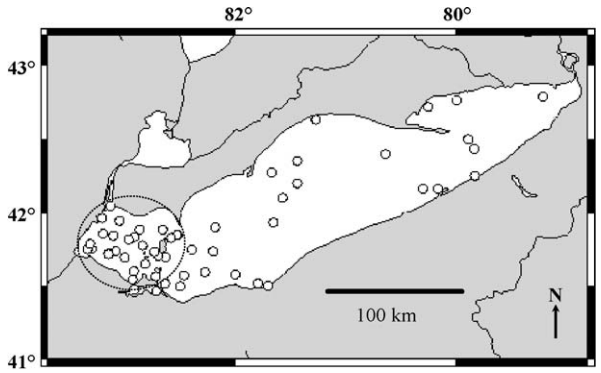

Fig. 1. Sampling locations in Lake Erie during the 2003–2005 field seasons. Samples were collected from stations in all three basins, with a primary focus on the western basin (circled by a dashed line).

3. Results

The stations which were occupied in Lake Erie during the 2003–2005 sampling are indicated on the map (Fig. 1). Environmental data collected from the stations are summarized in Tables 1 and 2. The estimated cell abundances in samples collected from different locations in western basin in the summer seasons of 2003–2005 are presented in Fig. 2 and discussed further below. Although toxic *Microcystis* is now known to have spread throughout the lake, the most frequent *Microcystis* blooms in the past decade have been within the western basin (Brittain et al., 2000; Rinta-Kanto et al., 2005; Ouellette et al., 2006). Therefore, we analyzed the data covering the entire lake, but we

Table 1
Raw data summary.

Date		Cyanobacterial 16S rDNA (copies L^{-1})	*Microcystis* 16S rDNA (copies L^{-1})	*mcyD* (copies L^{-1})	Microcystin ($\mu g\,L^{-1}$)	pH	Surface temperature
2003							
June	MIN	$2.4\ (\pm0.1) \times 10^7$	$2.2\ (\pm2.1) \times 10^3$	$6.5\ (\pm2.4) \times 10^3$	3.1	n/a	16.0
	MAX	$6.1\ (\pm2.4) \times 10^9$	$4.6\ (\pm2.2) \times 10^4$	$1.0\ (\pm1.3) \times 10^4$	–		19.6
		$n = 4$	$n' = 2$; $n'' = 2$	$n' = 2$; $n'' = 2$	$n' = 1$		$n = 3$
July	MIN	$1.3\ (\pm0.0) \times 10^7$	$1.8\ (\pm0.2) \times 10^4$	$3.5\ (\pm2.4) \times 10^3$	0.007	n/a	22.5
	MAX	$4.1\ (\pm2.1) \times 10^8$	$4.9\ (\pm0.2) \times 10^7$	$2.9\ (\pm2.4) \times 10^6$	–		23.2
		$n = 4$			$n' = 1$		$n = 2$
Aug.	MIN	$1.7\ (\pm0.5) \times 10^7$	$6.2\ (\pm1.8) \times 10^4$	$3.7\ (\pm0.7) \times 10^4$	0.02	n/a	24.3
	MAX	$3.0\ (\pm0.5) \times 10^{10}$	$3.4\ (\pm0.5) \times 10^{10}$	$5.0\ (\pm2.4) \times 10^8$	14.0		25.1
		$n = 16$	$n = 17$; $n' = 17$; $n'' = 15$	$n' = 16$; $n'' = 14$	$n' = 10$		$n = 5$
2004							
July	MIN	$1.3\ (\pm0.9) \times 10^6$	$7.7\ (\pm6.2) \times 10^4$	$1.5\ (\pm0.4) \times 10^4$	0.004	7.7	20.4
	MAX	$2.6\ (\pm1.4) \times 10^{11}$	$1.2\ (\pm0.3) \times 10^8$	$1.4\ (\pm0.5) \times 10^6$	1.09	9.0	26.0
		$n = 28$	$n' = 25$; $n'' = 25$	$n' = 16$; $n'' = 16$	$n' = 6$	$n = 5$	$n = 21$
2004							
Aug.	MIN	$1.9\ (\pm0.7) \times 10^7$	$1.3\ (\pm0.5) \times 10^5$	$9.3\ (\pm0.9) \times 10^3$	1.2	n/a	20.8
	MAX	$4.2\ (\pm0.1) \times 10^9$	$7.3\ (\pm2.6) \times 10^8$	$8.2\ (\pm4.6) \times 10^6$	21.7		22.2
		$n = 14$	$n = 20$ $n' = 19$; $n'' = 19$	$n' = 13$; $n'' = 12$	$n' = 20$		$n = 15$
Sept.	MIN	$2.3\ (\pm1.2) \times 10^8$	$1.3\ (\pm0.4) \times 10^8$	$1.8\ (\pm1.1) \times 10^6$	n/a	n/a	n/a
	MAX	$5.4\ (\pm2.1) \times 10^9$	$2.4\ (\pm1.2) \times 10^9$	$4.3\ (\pm1.6) \times 10^7$,			
		$n = 13$					
2005							
July	MIN	$1.8\ (\pm0.7) \times 10^5$	$8.3\ (\pm3.3) \times 10^4$	$3.2\ (\pm1.0) \times 10^4$	0.003	6.2	24.8
	MAX	$4.9\ (0.6) \times 10^{10}$	$4.9\ (\pm1.4) \times 10^8$	$2.9\ (\pm0.6) \times 10^7$	0.2	8.4	27.4
		$n = 34$	$n' = 17$; $n'' = 9$	$n' = 10$; $n'' = 5$	$n' = 20$	$n = 26$	$n = 12$
Aug.	MIN	$5.4\ (\pm8.6) \times 10^7$	$5.1\ (\pm1.3) \times 10^4$	$1.4\ (\pm1.1) \times 10^4$	0.0004	7.4	23.1
	MAX	$4.6\ (\pm0.9) \times 10^{10}$	$1.1\ (\pm0.3) \times 10^9$	$1.5\ (\pm0.2) \times 10^7$	0.1	8.4	27.1
		$n = 55$	$n' = 53$; $n'' = 50$	$n' = 49$; $n'' = 39$	$n' = 38$	$n = 26$	$n = 38$

n = samples analyzed. n' = samples where results were positive, n'' = samples where gene copies were quantifiable (>25 gene copies per qPCR reaction). n' and n'' are presented only if they are different from n. Where only one data point is available it is shown as the minimum only.

Table 2
Nutrient data summary.

Year, month		NO_3 (mg L^{-1})	NH_3 (mg L^{-1})	SiO_2 (mg L^{-1})	SRP (mg L^{-1})	TP (F) (mg L^{-1})	TP (UF) (mg L^{-1})	TN (F) (mg L^{-1})	N:P (molar ratio)
July 2004 (n = 9)	MIN	0.2	0.1	0.1	0.0005	0.008	0.02	0.3	0.92
	MAX	2.4	0.3	0.9	0.03	2.5	2.4	2.7	134.4
July 2005 (n = 31)	MIN	0.009	0.005	0.1	0.001	0.004	0.006	0.4	19.3
	MAX	0.5	0.3	3.7	0.06	0.09	0.2	0.9	388.9
August 2005 (n = 37–41)	MIN	0.004	0.005	0.1	0.0007	0.005	0.01	0.02	2.78
	MAX	1.4	0.1	4.2	0.1	0.1	0.2	2.4	179.5

F = filtered sample, UF = unfiltered sample.

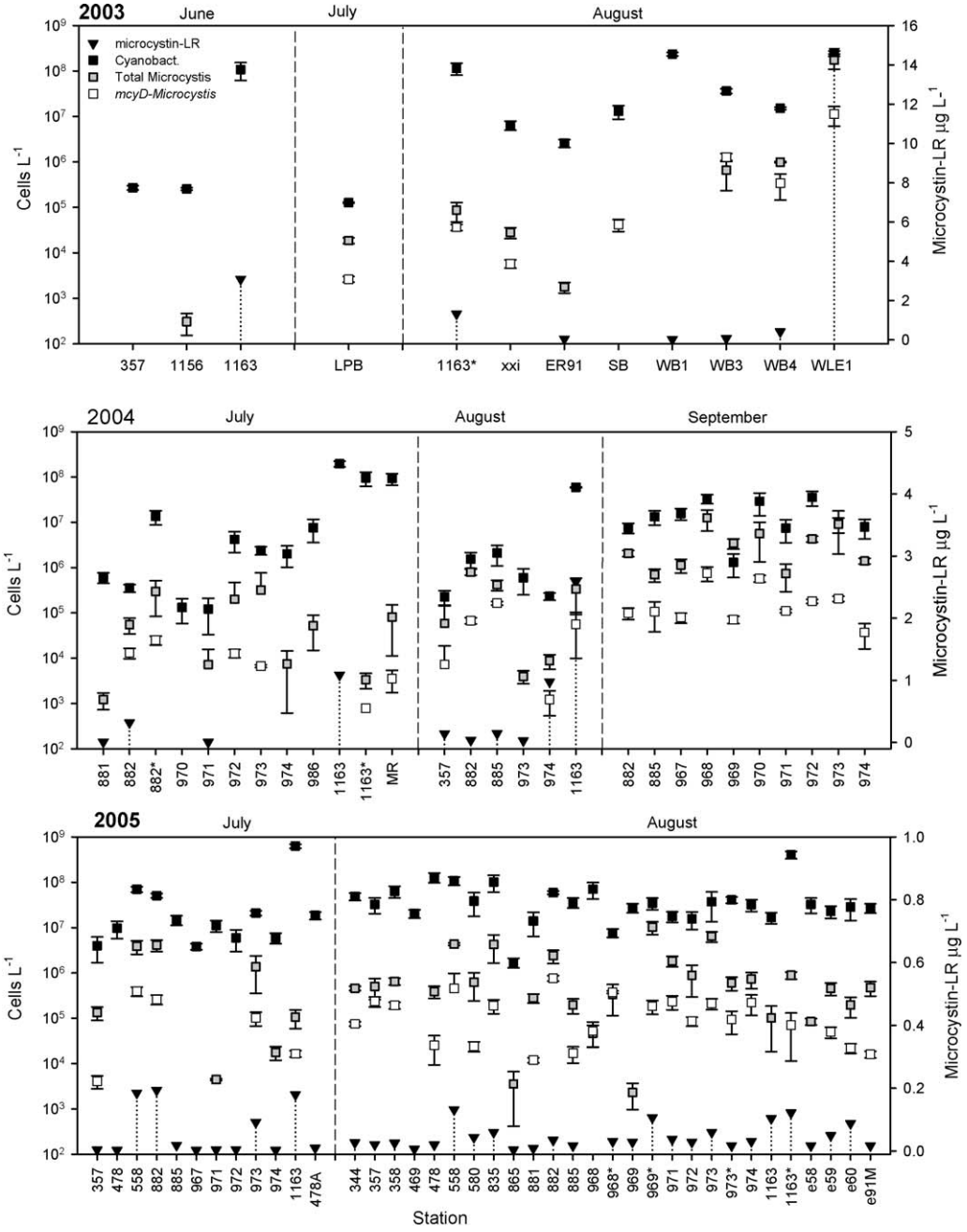

Fig. 2. Cell abundances (log transformed data) and microcystin-LR concentrations at western basin sampling locations in 2003–2005. Station numbers are organized according to their codes for each month. An asterisk mark indicates that same station was visited twice at separate times during one month.

Table 3
Relative abundances of total *Microcystis* and toxic *Microcystis* in Lake Erie.

Year	Month	% total *Microcystis* of total cyanobacteria		% toxic *Microcystis* of total cyanobacteria		% toxic genotypes of total *Microcystis*	
		Lake Erie	Western basin	Lake Erie	Western basin	Lake Erie	Western basin
2003	June	0.1 (0)	0.1 (0)	0	0	0	0
	July	5.9 (0.02 to 14.6)	14.6 (0)	1.2 (0.7 to 2.1)	2.1 (0)	19.5 (12.7 to 31.6)	14.2 (0)
	August	6.1 (0.04 to 71.4)	13.4 (0.07 to 71.4)	1.0 (0.01 to 4.7)	1.8 (0.03 to 4.7)	47.7 (6.6 to >100)	59.5 (6.6 to >100)
2004	July	5.0 (0.004 to 59.1)	4.3 (0.004 to 15.5)	0.8 (0.004 to 3.7)	0.7 (0.001 to 3.7)	41.5 (2.1 to >100)	11.4 (2.1 to 23.7)
	August	13.5 (0.08 to 51.7)	17.1 (0.6 to 51.7)	2.5 (0.1 to 7.8)	3.2 (0.1 to 7.8)	14.9 (4.3 to 39.3)	18.2 (8.4 to 39.3)
	September	41.7 (5.0 to >100)	49.1 (5.3 to >100)	1.5 (0.2 to 5.5)	1.7 (0.5 to 5.5)	6.5 (2.1 to 15.3)	6.9 (2.1 to 15.3)
2005	July	2.8 (0.02 to 8.1)	3.4 (0.02 to 6.5)	0.3 (0.003 to 0.6)	0.3 (0.003 to 0.6)	8.5 (3.1 to 15.6)	8.5 (3.1 to 15.6)
	August	3.1 (0.001 to 29.5)	3.7 (0.008 to 29.5)	0.5 (0.002 to 4.8)	0.6 (0.02 to 4.9)	17.7 (0.7 to >100)	22.5 (1.8 to >100)

The relative abundances were calculated using qPCR data converted to cells per liter. As most blooms occurred in the western basin this group is also shown separated from the whole lake data. Results are given as mean (minimum–maximum) for each month. Where maximum values were >100%, real qPCR estimates were used (and not 100%) so that variations in qPCR efficiency were normalized across the entire data set.

also separately examined the data for the western basin so that any anomalies might become apparent.

3.1. Community composition

Cell abundance data, determined by qPCR, were used to determine the relative abundances of total *Microcystis* and toxic *Microcystis* (Table 3). Based on monthly means in the western basin samples, *Microcystis* compromised between 0.1 and 49% percent of all the cyanobacterial cells, and within *Microcystis* population 0–60% of the cells were potentially toxin-producing genotypes. In the dataset including all samples, the corresponding percentages were 0.1–42% and 0–48%, respectively.

3.2. Correlations between microcystin concentration and abundance of cyanobacteria, Microcystis genotypes and limnological parameters

The Spearman Rank Correlation coefficient (r_s), number of pairs of data points (n) and probability values (P) for each correlation are presented in Table 4. In both data sets microcystin concentration yielded a positive correlation with the abundance of *Microcystis* 16S rDNA and *mcyD* copies. In the entire lake data set microcystin concentration also showed a significant positive correlation with the cyanobacterial 16S rDNA copy abundance. When using the estimated cell abundance data, a significant positive correlation was found between microcystin concentration and the relative abundance of total and toxic *Microcystis* cells among all cyanobacteria (Table 4); however, the percentage of toxic genotypes in the *Microcystis* population was not correlated with microcystin concentration. In both data sets the phytopigments phycocyanin and total chlorophyll-*a* concentrations yielded significant positive correlations with the toxin. Microcystin concentrations were also positively correlated with TP ($P < 0.05$), and negatively with TN:TP ratio. Additionally, in the entire lake data set microcystin correlated positively with SiO_2 and SRP.

3.3. Correlation of limnological parameters with 16S rDNA, Microcystis 16S rDNA and mcyD gene copies

The Spearman Rank Correlation coefficient (r_s), number of pairs of data points (n) and probability values (P) for each correlation are presented in Table 5 (entire lake data set) and Table 6 (western basin data set). In both data sets *Microcystis*-16S rDNA and *mcyD* copy abundance showed significant positive correlation with the abundance of cyanobacterial 16S rDNA copies. Also the total concentrations of phytopigments, chlorophyll-*a* and phycocyanin, were positively correlated ($P < 0.05$) with the copy abundances of each target gene.

In the entire lake data set the abundance of each target gene was positively correlated ($P < 0.05$) with TP (dissolved and

Table 4
Spearman correlation coefficients (r_s), n and P values for correlations with microcystin. Values considered significant ($P < 0.05$) are in boldface.

	Lake Erie			Western Basin only		
	r_s	n	P	r_s	n	P
Cyanobacterial 16S rDNA, copies L^{-1}	0.3266	88	**0.0019**	0.1423	41	0.3749
Microcystis 16S rDNA, copies L^{-1}	0.4016	70	**0.0006**	0.5772	39	**0.0001**
mcyD, copies L^{-1}	0.3080	58	**0.0187**	0.5766	34	**0.0004**
% cyanobacteria that are *Microcystis*	0.4233	69	**0.0003**	0.6965	37	**0.0000**
% cyanobacteria that are toxic *Microcystis*	0.3378	58	**0.0095**	0.5907	29	**0.0007**
% *Microcystis* that are toxic	0.1159	57	0.3907	0.0318	29	0.8700
Total chl *a*, μg L^{-1}	0.6608	85	**0.0000**	0.3831	40	**0.0147**
Phycocyanin, μg L^{-1}	0.7633	45	**0.0000**	0.6274	25	**0.0008**
pH	−0.1566	46	0.2986	−0.3086	27	0.1173
Surface water temperature, °C	−0.0296	67	0.8119	0.0182	34	0.9187
Water temperature at sampling depth	−0.0579	73	0.6266	−0.0656	34	0.7125
NO_3 (dissolved), mg L^{-1}	−0.1778	46	0.2370	−0.0958	25	0.6487
NH_3 (dissolved), mg L^{-1}	−0.1548	38	0.3534	0.0856	22	0.7049
SiO_2 (dissolved), mg L^{-1}	0.3526	49	**0.0130**	−0.0079	28	0.9680
SRP (dissolved), mg L^{-1}	0.4241	48	**0.0027**	0.1915	27	0.3386
TP (dissolved), mg L^{-1}	0.6907	49	**0.0000**	0.5475	28	**0.0026**
TP (part.+diss.), mg L^{-1}	0.7505	49	**0.0000**	0.5929	28	**0.0009**
TN (dissolved), mg L^{-1}	−0.0725	48	0.6242	0.1202	28	0.5425
TN:TP	−0.6363	48	**0.0000**	−0.4048	28	**0.0326**

Table 5
Spearman correlation coefficients (r_s), n and P values for correlations with target gene abundances in the whole lake data set. Values considered significant ($P < 0.05$) are in boldface.

	Cyanobacterial 16S rDNA, copies L^{-1}			*Microcystis* 16S rDNA, copies L^{-1}			*mcyD*, copies L^{-1}		
	r_s	n	P	r_s	n	P	r_s	n	P
Microcystis 16S rDNA, copies L^{-1}	0.5811	143	**0.0000**						
mcyD, copies L^{-1}	0.3877	118	**0.0000**	0.7934	119	**0.0000**			
Total chl *a*, μg L^{-1}	0.5906	146	**0.0000**	0.5008	126	**0.0000**	0.6242	103	**0.0000**
Phycocyanin	0.7771	46	**0.0000**	0.5183	39	**0.0007**	0.3362	36	**0.0450**
pH	0.1321	57	0.3271	−0.1113	44	0.4718	−0.3244	39	**0.0439**
Surface water temperature, °C	0.3324	112	**0.0003**	0.0661	104	0.5049	0.1789	89	0.0935
Water temperature at sampling depth	0.3926	116	**0.0000**	0.0250	96	0.8086	0.0661	74	0.5759
NO_3, mg L^{-1}	−0.3359	78	**0.0026**	−0.4039	58	**0.0017**	−0.3840	53	**0.0045**
NH_3 (dissolved), mg L^{-1}	−0.3497	65	**0.0043**	−0.3365	51	**0.0158**	−0.2813	47	0.0554
SiO_2 (dissolved), mg L^{-1}	0.3184	81	**0.0038**	0.1533	61	0.2382	0.1242	56	0.3617
SRP (dissolved), mg L^{-1}	0.2622	80	**0.0188**	0.1313	60	0.3172	0.1569	55	0.2526
TP (dissolved), mg L^{-1}	0.4706	81	**0.0000**	0.4607	61	**0.0002**	0.5425	56	**0.0000**
TP (particulate), mg L^{-1}	0.5706	81	**0.0000**	0.4718	61	**0.0001**	0.5596	56	**0.0000**
TN (dissolved), mg L^{-1}	−0.3143	80	**0.0045**	−0.3451	60	**0.0069**	−0.3412	55	**0.0108**
TN:TP ratio	−0.4799	80	**0.0000**	−0.4753	60	**0.0001**	−0.5664	55	**0.0000**

Table 6
Spearman correlation coefficients (r_s), n and P values for correlations with target gene abundances in the western basin data set. Values considered significant ($P < 0.05$) are in boldface.

	Cyanobacterial 16S rDNA, copies L^{-1}			*Microcystis* 16S rDNA, copies L^{-1}			*mcyD*, copies L^{-1}		
	r_s	n	P	r_s	n	P	r_s	n	P
Microcystis 16S rDNA, copies L^{-1}	0.4300	54	**0.0012**						
mcyD, copies L^{-1}	0.4641	47	**0.0010**	0.7641	47	**0.0000**			
Total chl *a*, μg L^{-1}	0.5379	45	**0.0001**	0.5025	42	**0.0007**	0.4575	36	**0.0050**
Phycocyanin	0.7731	25	**0.0000**	0.5321	23	**0.0090**	0.4657	23	**0.0251**
pH	−0.2917	27	0.1398	−0.1864	25	0.3722	−0.2089	24	0.3272
Surface water temperature, °C	0.4933	41	**0.0010**	0.1155	38	0.4898	0.0022	32	0.9904
Water temperature at sampling depth	0.2564	41	0.1057	−0.0616	38	0.7131	−0.1341	32	0.4642
NO_3 mg L^{-1}	−0.3897	25	0.0542	−0.3532	23	0.0983	−0.3367	21	0.1356
NH_3 (dissolved), mg L^{-1}	0.0397	22	0.8608	−0.2583	21	0.2582	−0.0838	19	0.7331
SiO_2 (dissolved), mg L^{-1}	0.4036	28	**0.0332**	0.2447	26	0.2284	0.0900	24	0.6759
SRP (dissolved), mg L^{-1}	0.2655	27	0.1807	0.1485	25	0.4787	0.1139	23	0.6047
TP (dissolved), mg L^{-1}	0.2389	28	0.2207	0.3186	26	0.1127	0.4870	24	**0.0158**
TP (particulate), mg L^{-1}	0.2272	28	0.2450	0.3634	26	0.0680	0.4931	24	**0.0143**
TN (dissolved), mg L^{-1}	−0.2425	28	0.2138	−0.1605	26	0.4336	−0.2245	24	0.2917

dissolved + particulate fractions) and negatively correlated ($P < 0.05$) with NO_3, TN and TN:TP ratio. *Microcystis* and cyanobacterial 16S rDNA copies showed strong negative correlation also with NH_3, while pH correlated negatively only with the abundance of *mcyD* gene copies. In addition, in the entire lake data set cyanobacterial 16S rDNA copies showed significant positive correlations with SiO_2, SRP concentrations and water temperature.

When we constrained our observations to the western basin data set, fewer strong correlations with environmental parameters were found. The abundance of *Microcystis* 16S rDNA and *mcyD* copies correlated positively with TP (particulate) while *mcyD* copies correlated also positively ($P < 0.05$) with TP (dissolved and dissolved + particulate fractions). Cyanobacterial 16S rDNA copies yielded a significant negative correlation ($P < 0.05$) with NO_3 and a significant positive correlation with surface water temperature and SiO_2.

4. Discussion

To elucidate the linkage between *Microcystis* population dynamics and microcystin concentrations we carried out studies over three summer seasons in Lake Erie. During the study period *Microcystis* formed a portion of the total cyanobacterial community in each year. In two of the seasons (2003 and 2004) relatively high microcystin concentrations (up to 22 μg L^{-1}) were observed, while in the third season (2005) microcystin concentrations were generally low (up to 0.2 μg L^{-1}). As such this survey over three seasons brackets the conditions associated with bloom promoting and non-promoting years. Because not all chemical analyses were performed on each sample, the correlations presented here are based on analyses of subsets of the data.

Our data demonstrate that in Lake Erie, microcystin-producing *Microcystis* genotypes generally coexist with non-toxic genotypes. In the entire cyanobacterial population, the proportion of microcystin-producing *Microcystis* cells did not exceed 8% of the total cyanobacterial abundance in any of the samples analyzed. Our results generally agree with observations from previous studies, in spite of the use of different sampling approaches and obtaining samples from different locations, in that the relative abundance of potentially toxic *Microcystis* commonly remains well below 100% of total *Microcystis* abundance in natural populations. Kurmayer and Kutzenberger (2003) estimated the proportion of

toxic genotypes within colonies of *Microcystis* to be 1.7–71% in samples collected from Lake Wannsee (Germany), while in Lake Mikata (Japan) potentially toxic genotypes formed 0.5–35% of the total *Microcystis* population (Yoshida et al., 2007).

Although qPCR has now been widely accepted as a tool to analyze the genotypic composition of cyanobacterial communities, one of the pitfalls of the method is the difficulty of converting the gene quantities to quantities of cells that carry these target genes, which makes the enumeration of cyanobacterial cells in natural samples by qPCR somewhat error prone. Reflecting this difficulty some samples analyzed in this study showed unrealistic results as the abundance of potentially toxic *Microcystis* appeared >100% total *Microcystis* abundance (August 2003 (1 sample), July 2004 (3 samples); August 2005 (1 sample)) and the abundance of *Microcystis* cells appeared >100% of the total abundance of cyanobacteria (September 2004, one sample). These anomalous results may arise from the use of the 16S rDNA as a target for quantification of both cyanobacteria and *Microcystis* due to the variable copy number of 16S rDNA operons or their sequence heterogeneity in cyanobacterial cells in natural populations (Crosby and Criddle, 2003), or the error in estimating the size of the genome in reference strains (Kardinaal and Visser, 2005).

In Lake Erie microcystin concentrations show strong positive correlation with the abundance of cyanobacterial and *Microcystis* 16S rDNA as well as *mcyD* copy abundance. Also phytopigments chlorophyll-*a* and phycocyanin show strong positive correlation with microcystin concentrations. These observations support previous suggestions (Rinta-Kanto and Wilhelm, 2006) that *Microcystis* is the most significant contributor to the microcystin concentrations measured in Lake Erie. In the western basin of Lake Erie the presence of other cyanobacteria (potential microcystin producing genera, e.g. *Planktothrix* (Rinta-Kanto and Wilhelm, 2006) and potential producers of other toxins such as *Anabaena* and *Aphanizomenon* sp. (unpublished data) as well as non-toxic ones such as *Synechococcus* sp. (Ouellette et al., 2006)) may explain the lack of correlation between microcystin concentrations and the abundance of cyanobacterial 16S rDNA copies in the subset of the data. Indeed the most abundant cyanobacterial cell type in the lake are *Synechococcus* (or *Synechococcus*-like) cells that are commonly ignored by microscopists but are readily apparent in molecular analyses (Wilhelm et al., 2006).

In both of our data sets the relative abundance of toxic *Microcystis* cells among cyanobacteria correlated with microcystin concentrations, whereas no such relationship was found between microcystin concentrations and the proportion of potentially toxic genotypes among total *Microcystis* cells. This was especially true in 2005 when relatively low microcystin concentrations were recorded throughout the summer. At the same time the mean percentage of potentially toxic genotypes varied between 8.5–17.7% (entire lake) or 8.8–22.5% (western basin) in corresponding samples. Our data agree with a recent study of three Dutch lakes (Lake 't Joppe, Lake Kinselmeer and Lake Volkerak) by Kardinaal et al. (2007) who also found that microcystin concentrations correlated roughly with the biovolume of *Microcystis* while the relationship between the relative abundance of potentially toxic genotypes and microcystin concentrations was less prominent.

In the entire lake data set, microcystin concentration and copy abundance of *Microcystis* 16S rDNA and *mcyD* showed significant negative correlations between TN:TP and TN, and positive correlations with TP. In the western basin microcystin concentration and *mcyD* copy abundance were negatively correlated with TN:TP ratios and positively with TP, suggesting that in Lake Erie increasing phosphorus concentrations can increase both the abundance of *Microcystis* as well as microcystin production. Concentrations of inorganic nitrogen (TN, NO_3, NH_3) correlated negatively with the abundance of *Microcystis* 16S rDNA and *mcyD* copies (TN, NO_3) whereas these nutrients showed no correlation with microcystin concentrations. Furthermore, in the entire lake data set, pH correlated negatively with *mcyD* abundance. Previous studies have demonstrated that nitrogen and phosphorus concentrations and their relationship are significant factors affecting *Microcystis* biomass and microcystin production in natural systems (Sivonen and Jones, 1999; Kardinaal and Visser, 2005; Gobler et al., 2007), however the importance of each of these factors may vary strongly between different environments. Our observations agree best with those from a study conducted in small, eutrophic lakes in Canada (Kotak et al., 2000), where a positive correlation with TP, *Microcystis* cellular biomass and microcystin was found. In the same study *Microcystis* biomass was negatively correlated with the ratio of TN and TP, and inorganic nitrogen. In contrast with our findings regarding the *mcy*-gene copy abundance, Yoshida et al. (2007) found a positive correlation between the abundance of *mcyA*-copies and nitrate but no correlation with *mcyA* abundance and orthophosphate concentrations in a Japanese lake. The correlation of *Microcystis* and microcystin concentrations with TP supports the hypothesis that factors increasing the growth rate of the cells also facilitate microcystin production allowing maximal toxin production at periods of maximal growth (Orr and Jones, 1998; Lee et al., 2000; Gobler et al., 2007). Interestingly, the relative abundance of potentially toxic *Microcystis* cells in the *Microcystis* population does not seem to follow this pattern. Although the mechanisms governing microcystin production at the cellular level are not known yet, these findings strongly suggest that such mechanisms are important.

Contrary to our data, positive correlations between pH and the biomass of total *Microcystis* and cellular microcystin content in natural populations have been observed previously, as cyanobacteria are able to outcompete other phytoplankton species under high pH conditions with the help of their efficient carbon concentration mechanisms (Wicks and Thiel, 1990; Kotak et al., 2000; Rantala et al., 2006). Microcystin concentrations in the entire lake data set also correlated positively with SRP, SiO_2 and with cyanobacterial 16S rDNA copies, although they did not correlate with any of the *Microcystis* genotypes. Correlation with SRP indicates that different P-species may elicit differential effects on proliferation and microcystin production in subpopulations of cyanobacteria in Lake Erie. Similar association with silicate and cyanotoxins (microcystin and nodularin) has been observed in previous studies (Repka et al., 2004; Aboal et al., 2005). Silica can also be released in to the water column from sediments or from external sources and it can thus be a sign of external nutrient inputs in the system which could in turn affect microcystin concentrations. This could also be an indication of the sedimentary input of materials that includes *Microcystis* seed populations that could result in a bloom (Rinta-Kanto et al., 2009) although at this juncture the point remains somewhat speculative.

One important caveat is that the role of zooplankton grazing has not been accounted for in this study. Grazers may affect the total biomass of cyanobacteria and *Microcystis*, as well as the relative abundance of *Microcystis* genotypes because grazers may also exhibit selective grazing depending on whether the cell is producing toxin or not (Gobler et al., 2007). It is thus possible that some parameters associated with grazing may have correlated with toxic vs. non-toxin producing genotypes. As part of future efforts this aspect of *Microcystis* ecology should no doubt be further investigated.

To our knowledge this is the first large-scale and multi-year study offering insight into factors influencing the *Microcystis* population and microcystin production in a large lake system. Our data set is unique in that it spans the known range of *Microcystis* bloom events for Lake Erie, allowing for a thorough profile of the conditions associated with bloom and non-bloom events. While

this type of study may not highlight factors that become evident when analyzing *Microcystis* in cultures or natural populations on small scale (e.g. at a single specific sampling location or in a more homogenous small-lake environment) the results of our study highlight some of the key factors influencing *Microcystis* dynamics over a wide spatial and temporal scale. Most notably, our findings suggest that in Lake Erie increased phosphorus loading from autochthonous or allochthonous sources may be the reason for the recurring *Microcystis* blooms in recent years.

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